Quantification of the Physical Robustness of Lyophilized Biotherapeutics

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1 Abstract

For many decades, lyophilization (freeze-drying) has been the method of choice for the stabilization of labile drugs, biotherapeutics and vaccines, as it is considered a gentle process compared with traditional drying methods (such as spray drying, oven drying, fluidized bed drying). Lyophilization also provides an opportunity to produce material with low moisture content and high surface area, allowing the possibility of long-term stability at ambient temperatures and rapid reconstitution prior to use. However, due to their low density, lyophilized products can undergo physical breakup during transportation and handling, sometimes becoming fragmented and powdery, which in turn can have an impact on end user perception of product quality as well as the time taken for reconstitution.

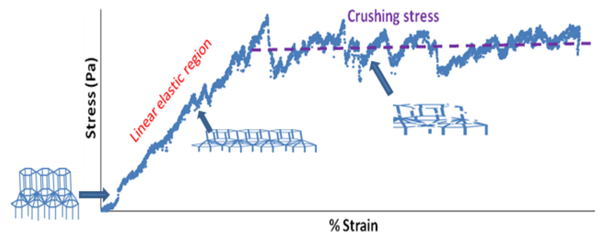
There are a number of standard quantitative tests for critical quality attributes (CQAs) such as residual moisture (or solvent) levels, activity, thermal properties and stability, but while most manufacturers would agree that cosmetic appearance of the product is also important, traditionally, there has been no method to quantify this aspect of the product. Techniques such as scanning electron microscopy (SEM) can provide an idea of morphology at the microscopic level, while gas adsorption methods can go one step further in providing an estimate of specific surface area and mean pore diameter of a lyophile; however, it can legitimately be argued that the sample preparation process itself for either of these measurements can lead to changes to the morphology of the material under test. Rheometers and tensile testing devices on the market are generally designed for application to higher density and less flexible materials than lyophiles, and with some degree of sample preparation required.

In this study, we developed a customized testing device comprising a load cell, linear actuator, indenter and control software in order to measure the mechanical properties of freeze-dried materials in situ, thus circumventing the need for sampling. Vials of mannitol, sucrose, trehalose, dextran, model proteins and various combinations of these components were lyophilized under different processing conditions (temperatures, ramp rates, chamber pressures) and from a series of starting concentrations, to provide a realistic range of samples for testing the sensitivity of the 'MicroPress' instrument and repeatability of measurements.

2 Materials & Methods

A range of excipients, excipient mixtures and model protein formulations at a series of concentrations and molecular ratios were lyophilized under a number of different freezing and sublimation conditions in glass vials, using a VirTis AdVantage EL or Genesis 25EL freeze-dryer (SP Scientific, Gardiner NY). A device was built using a standard load cell and linear actuator combined with a customized indenter and control software (Fig. 1). The resulting instrument is able to provide a 1 gram force at 0.01mm steps into freeze-dried materials. Samples were subjected to stress-strain measurements in situ (without the need to remove them from the vials). Young's Modulus was taken as the gradient of the plot of stress vs. strain in the linear elastic region, and the failure point defined at the point of the gradient suddenly changing to zero, indicating crushing (Fig. 2). All tests were carried out within 3 minutes, in order to limit atmospheric moisture uptake by the lyophilized materials, which can alter their mechanical properties by plasticization (Ref. 1).

Fig. 1: Customized compression tester (MicroPress)



$E = \frac{\sigma}{\varepsilon}$ where σ is the stress and ε is the strain, and E is the Young's / Elastic modulus

Fig. 2: Example of a stress/strain profile

3 Results & Discussion

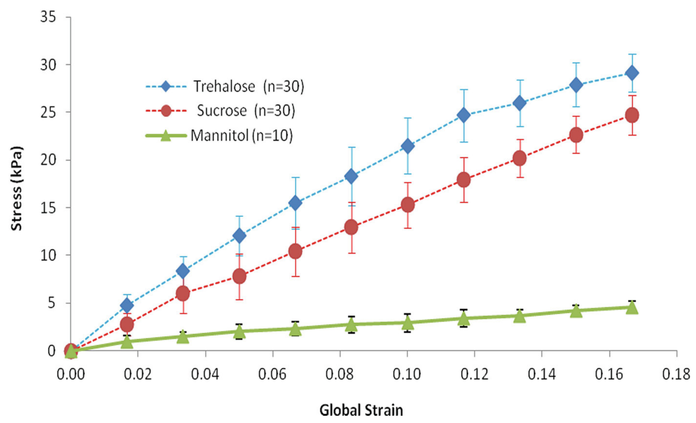


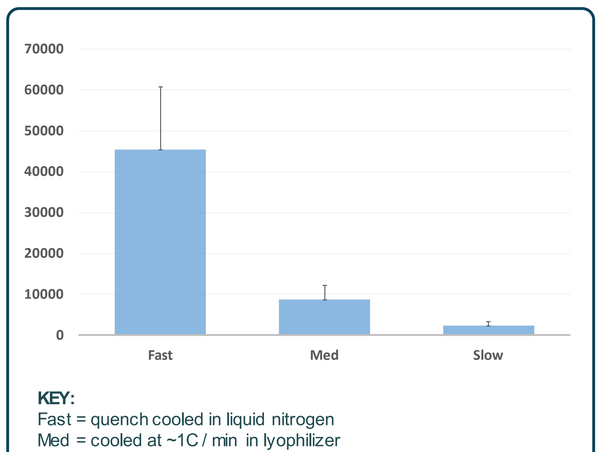
Fig. 3: Mean maximum stress-strain curves for excipients processed using identical conditions. Freeze-drying runs were carried out in triplicate for trehalose and sucrose (n=10 per run), single run for mannitol.

Experimental data indicate that:

Excipients freeze-dried under identical conditions from the same starting concentration of 5% (w/v) can display markedly different mechanical properties (Fig. 3), even when the outward appearance of the cakes is similar with no obvious visible defects;

Freezing conditions have a pronounced effect on the properties of resulting mannitol cakes (Fig. 4), which may be related to ice crystal size (affecting porosity) but also possibly to inherent properties related to polymorph type that can result from the application of different cooling rates and/or the application of annealing during the cycle; this is supported by evidence from x-ray diffractograms that show that different polymorphs had been created (Fig. 5);

This method appears to have sufficient sensitivity to detect subtle differences in mechanical properties between neighboring vials of identical material from a single lyophilizer shelf (Fig. 6), thus indicating when cycle conditions might need to be optimized in order to reduce intra-batch variability. Results also demonstrated that both Young's Modulus and strength increase near-linearly with density (data not shown), which may assist in the optimization of the starting concentration vs. fill volume for a particular formulation (Ref. 2).



KEY:
Fast = quench cooled in liquid nitrogen
Med = cooled at ~1C / min in lyophilizer
Slow = cooled at <0.25C / min in lyophilizer

Fig. 4: Young's Modulus data (Pa) for Mannitol as a function of cooling rate employed in lyophilisation cycle

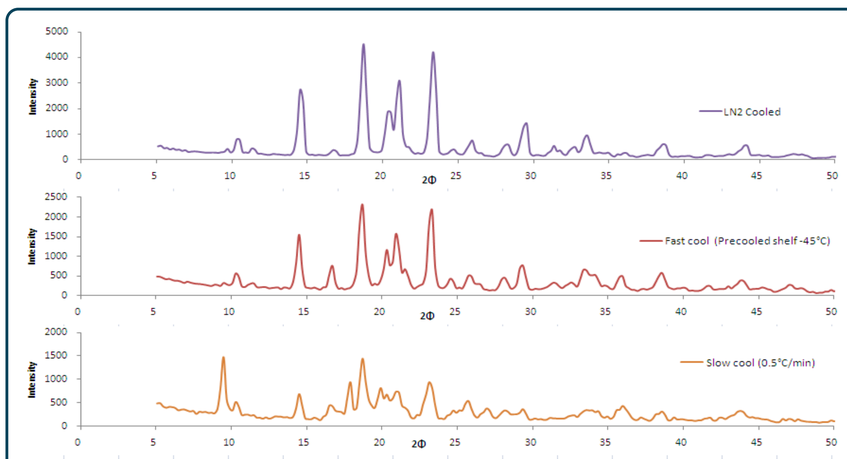


Fig. 5: XRD analysis of mannitol samples indicate that different polymorphs were produced. The diffractogram of the 'Slow cool' sample exhibits a notable peak at a value of 10 (2θ) which is characteristic of the δ polymorph, while the two upper diffractograms for samples cooled more rapidly are both typical of β and α polymorphs.

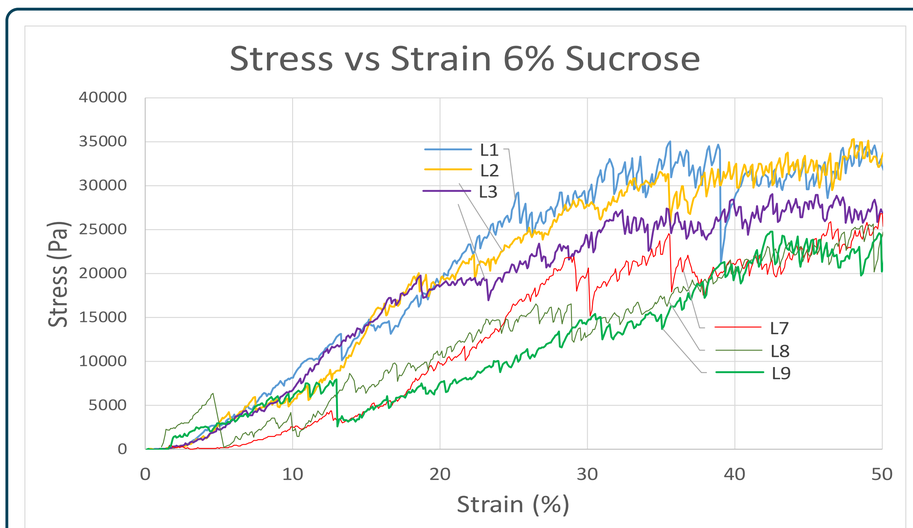
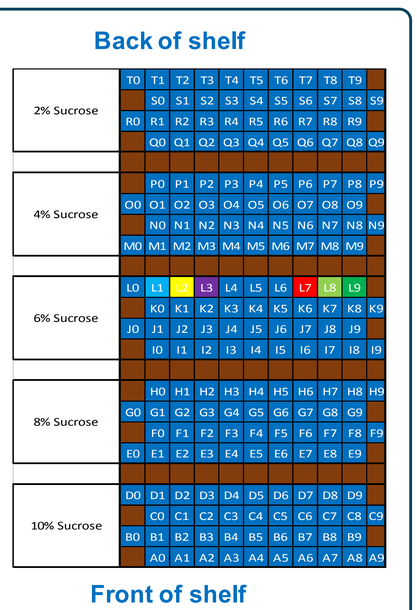


Fig. 6: Differences in mechanical properties observed for 6% sucrose samples across a single shelf



4 Conclusion

Data demonstrate that the customized instrument is sufficiently sensitive to detect statistically significant differences in the mechanical properties of single ingredients when lyophilized individually, that excipients that appear to have similar cake-forming properties have markedly different inherent Young's Modulus and strength values, both of which increase near-linearly with solute density.

Significant differences were detectible for samples of mannitol where different freezing conditions were employed in the lyophilization cycle, indicating that not only is the mean pore diameter hugely influential on the mechanical properties of the resulting lyophile, but also that amorphous / crystalline behavior and possibly even polymorphism could have a measurable impact.

With further optimization of the instrument parameters, differences were even detected in the mechanical properties of lyophiles in vials taken from different locations across a single lyophilizer shelf.

We therefore believe that this method could represent a valuable addition to the existing array of techniques available to provide quantitative measurement of lyophile critical quality attributes (CQAs).

5 References and Acknowledgments

Ref. 1: S. McCartney Eng. Doc. thesis "Mechanical Characterisation of Freeze-Dried Biopharmaceuticals", Imperial College London (2014).

Ref. 2: S. Hedberg, S. Devi, A. Duralliu and D. Williams, "Mechanical Behavior and Structure of Freeze-Dried Cakes", In: K. Ward and P. Matejtschuk (eds.), Lyophilization of Pharmaceuticals and Biologicals: New Technologies and Approaches, Springer NY (in press).

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